Neurobiology of Aging 35 (2014) 1780.e1–1780.e5

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Detecting gene mutations in Japanese Alzheimer's patients by semiconductor sequencing

Ryoichi Yagi [a,b], Ryosuke Miyamoto [a,c], Hiroyuki Morino [a], Yuishin Izumi [c], Masahito Kuramochi [a], Takashi Kurashige [d], Hirofumi Maruyama [a], Noriyoshi Mizuno [b], Hidemi Kurihara [b], Hideshi Kawakami [a,*]

[a] Department of Epidemiology, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan
[b] Department of Periodontal Medicine, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan
[c] Department of Clinical Neuroscience, Institute of Health Biosciences, Graduate School of Medicine, University of Tokushima, Tokushima, Japan
[d] Department of Anatomical Pathology, Hiroshima University Hospital, Hiroshima, Japan

ARTICLE INFO

Article history:
Received 22 July 2013
Received in revised form 16 December 2013
Accepted 21 January 2014
Available online 25 January 2014

Keywords:
Alzheimer's disease
PSEN1
PSEN2
APOE
Mutations
Ion sequencing technology

ABSTRACT

Alzheimer's disease (AD) is the most common form of dementia. To date, several genes have been identified as the cause of AD, including *PSEN1*, *PSEN2*, and *APP*. The association between *APOE* and late-onset AD has also been reported. We here used a bench top next-generation sequencer, which uses an integrated semiconductor device, detects hydrogen ions, and operates at a high-speed using nonoptical technology. We examined 45 Japanese AD patients with positive family histories, and 29 sporadic patients with early onset (<60-year-old). Causative mutations were detected in 5 patients in the familial group (11%). Three patients had a known heterozygous missense mutation in the *PSEN1* gene (p.H163R). Two patients from 1 family had a novel heterozygous missense mutation in the *PSEN1* gene (p.F386L). In the early onset group, 1 patient carrying homozygous *APOEε4* had a novel heterozygous missense mutation in the *PSEN2* gene (p.T421M). Approximately 43% patients were *APOEε4* positive in our study. This new sequencing technology is useful for detecting genetic variations in familial AD.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by amnesia, poor judgment, impaired visuospatial abilities or language functions, and changes in personality. (McKhann et al., 2011) Neuronal loss, senile plaques, and neurofibrillary tangles are the pathologic hallmarks of AD. To date, several genes have been identified as the cause of AD, including *PSEN1* (MIM 104311) (Sherrington et al., 1995), *PSEN2* (MIM 600759) (Levy-Lahad et al., 1995), and *APP* (MIM 104760) (Goate et al., 1991). These genes are involved in the amyloid-beta pathway, and have been included as causative genes in the new diagnostic guidelines for AD (Jack et al., 2011). *PSEN1* mutations are the most frequent, whereas *PSEN2* mutations are very rare. The presenilin 1 and 2 proteins are components of the γ secretase complex. *APP* encodes the amyloid precursor protein, and mutations are mainly adjacent to the ß and γ secretase cleavage site (Cohn-Hokke et al., 2012). *APOE* (MIM 107741) has also been associated with late onset AD because the frequency of *APOEε4* allele was found to be high in these patients (Saunders et al., 1993). *APOE* was proposed to be a moderately penetrant gene with semi-dominant inheritance (Genin et al., 2011).

Until recently, these genes were screened using Sanger sequencing. However, Sanger sequencing is time-consuming and costly; therefore, it is not ideal for routine diagnostic purposes. Over the past few years, high-throughput genome technologies have changed the genetic landscape of AD (Bettens et al., 2013). We used ion-sequencing technology in the present study. Ion sequencing uses an integrated semiconductor device to detect hydrogen ions and operates at high-speed using nonoptical technology. We also assessed the disadvantages of this technology.

2. Methods

2.1. Subjects

Participants were diagnosed with probable AD by neurologists. We tested 45 patients with a positive family history of dementia (familial group, age of onset; 40–82 years). Although most cases showed a dominant inheritance, some patients had incomplete

* Corresponding author at: Department of Epidemiology, Hiroshima University, Hiroshima, Japan. Tel.: +81 82 257 5846; fax: +81 82 257 5848.
E-mail address: hkawakam@hiroshima-u.ac.jp (H. Kawakami).

0197-4580/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2014.01.023

penetrance. In addition, we enrolled 29 patients with early onset (<60-year-old) AD who had no family history (early onset group, age of onset; 39–59 years). New variations were also analyzed in 112 Japanese control subjects and control exome data were obtained from 147 patients undergoing exome analysis for diseases other than AD (in-house exome). The research procedure was approved by the Ethics Committee of Hiroshima University. All examinations were performed after obtaining written informed consent from the patients or their families.

2.2. Sequencing

Genomic DNA was extracted from the peripheral lymphocytes of the patients. Sixty-seven primers pairs for the exonic regions of *PSEN1*, *PSEN2*, *APP*, and *APOE* were designed by the Ion AmpliSeq Designer (https://www.ampliseq.com/browse.action) as 2 subgroups; Primer pool 1 for 35 amplicons and primer pool 2 for 32 amplicons (Supplementary Table 1). Four regions were not covered by originally primer design (Supplementary Table 2), and the uncovered regions are amplified by polymerase chain reaction (PCR) respectively, and sequenced directly by Sanger method.

We performed Ion Personal Genome Machine (PGM) sequencing according to the provided protocol of Ion Ampliseq Library Kit 2.0 (Life Technologies, Carlsbad, CA, USA) and the Ion PGM-200 sequencing supplies kit (Life Technologies). We examined amplicon coverage after ion PGM sequencing. The amplicons were validated with standard PCR-based amplification followed by sequencing analysis using an Applied Biosystems 3130 DNA sequencer (Life Technologies) in cases in which the amplicon missed covering the target region or the depth of amplicon coverage was below 20× reads. Variants were identified using TorrentSuite Software 3.2, and validated with standard PCR-based amplification followed by Sanger sequencing. The variants were assessed by prediction algorithms (i.e., Sorting Intolerant From Tolerant [http://sift.jcvi.org/], Polymorphism Phenotyping v2 [http://genetics.bwh.harvard.edu/pph2/], and Pmut [http://mmb2.pcb.ub.es:8080/PMut/]). We investigated whether a variant was novel by referring to the dbSNP137 (http://www.ncbi.nlm.nih.gov/SNP/), 1000 genomes (http://www.1000genomes.org/), and Alzheimer Disease & Frontotemporal Dementia Mutation (http://www.nolgen.ua.ac.be/ADMutations/) databases. Novel variations were judged to be mutations when more than 2 prediction algorithms that predict whether an amino acid substitution affects protein function were positive and when the variant was not detected in control subjects (Xiao et al., 2012).

2.3. Copy-number assay

APP alleles were quantified using a SYBR Green real-time PCR assay on the Applied Biosystems StepOnePlus System (Life Technologies). We designed probes to detect *APP* duplication in intron 1, exon 7, and exon 18 of the *APP* locus (Rovelet-Lecrux et al., 2006). We used the *GAPDH* as a control gene.

3. Results

3.1. Amplicon sequencing data

Table 1 shows the total sequencing outputs and mean read lengths from the 2 types of chips. The means of Ion 314 chip and Ion 318 chip based on >20× coverage were 93.9% and 93.7%, respectively. An average 2.62 amplicons per sample of depths of coverage below 20× reads were recognized, and we validated these by Sanger sequencing. The distribution of the mean coverage depth for all exon targets, displayed by amplicon, is shown in Supplementary Table 3. The average read depths of each gene revealed 572.5 in *APP*, 650.4 in *PSEN1*, 569.6 in *PSEN1*, and 425.9 in *APOE*, respectively. The mean coverage depth of each sample, is shown in Supplementary Table 4. The average read depth was 569.1 (min = 64.5, max = 2462.4).

Table 1
Average data generation per sample from the 2 types of chips

Chip	Number of reads	Bases	>= Q20 bases	Mean read length	Mean read depth
314	24654.98	2846887	2200083.49	116	310.38
318	71112.85	7650168	5573694.54	109	556.61

3.2. Variants in causative genes

We identified 5 heterozygous variations in 4 genes in familial cases (Table 2). Two variants were detected in the *PSEN1* gene. One was a known heterozygous missense mutation in the *PSEN1* gene (patient 1, 2, and 3: c.488 A>G, p.H163R, rs63750590). Another was a novel heterozygous missense variant in the *PSEN1* gene (patient 4: c.1158C>A, p.F386L, Fig. 1). The variant was determined to be pathogenic by 2 function-prediction algorithms, but was not detected in our in-house exome or the controls. The father was already deceased and had had dementia; the 80-year-old mother showed no signs of dementia and did not carry a variant of *PSEN1*. Two brothers of patient 4 also developed dementia. The elder brother carried the same variant, whereas the younger brother did not agree to the test (Fig. 1). Segregation analysis through the family also provided evidence that this variant was pathogenic.

A novel heterozygous variation of *PSEN2* (c.773C>T, p.A258V) was detected, but it was not predicted to be a pathologic variation by any prediction program. One variation in *APP* (c.1530G>C, p.K510N) was identified in the *APP* gene, but was detected in both a control subject and in one of our own in-house exome databases. Thus, the variation did not appear to be a pathogenic variant.

A novel heterozygous missense variation (c.1262C>T, p.T421M) was only detected in the *PSEN2* gene (patient 5) in the early onset group. This patient also carried *APOEε4* in the homoallelic state. We could not determine whether these variants were inherited from the parents because we could not obtain samples from the family.

Regarding the *APOE* gene, only *APOEε4* (c.471T>C, p.C130R, rs429358) was detected. The frequency of patients carrying *APOEε4* alleles was approximately 43%. No significant differences were observed between the early onset group (15/29; 52%) and familial group (17/45; 38%) ($p = 0.3365$).

3.3. APP duplication

Using real-time PCR, we confirmed that no patient with AD had a genomic *APP* duplication.

3.4. Clinical characteristics of patients

The clinical characteristics of the patients are shown in Table 3. All the patients who had gene mutations were early onset (40 to 55-year-old). Patients 2 and 3 were sisters. Patients 4 and 6 were sister and brother. Patient 5 was sporadic. More information on patient characteristics is provided in Table 3.

4. Discussion

We detected a new mutation in the *PSEN1* gene (c.1158C>A, p.F386L) and one known mutation in the *PSEN1* gene (c.488 A>G, p.H163R, rs63750590). The percentage of detected gene mutations